

**REMARKS**

In the present amendment, claims 21, 22, and 23 have been amended. Of the pending claims, claims 21, 22, and 23 are under consideration, and claims 1- 20 are withdrawn from consideration.

Applicants note that the claims have been amended to more clearly recite the presently claimed invention and to better comply with idiomatic English and standard U.S. practice. No new matter has been added.

**Response to claim objections**

Applicants respectfully note that in view of the amendments to the claims the present claim objections should be moot.

**Response to rejections under 35 U.S.C. § 112, second paragraph**

The Office Action rejects claim 21 under 35 U.S.C. § 112, second paragraph as allegedly being indefinite.

In response, Applicants respectfully note that in an attempt to advance prosecution and without expressing agreement with or acquiescence to the rejection, claim 21 has been amended to better clarify the presently claimed invention. In view of the present claim amendments, withdrawal of the indefiniteness rejections is respectfully requested.

**Response to rejections under 35 U.S.C. § 112, first paragraph**

The Office Action rejects claims 21-23 under 35 U.S.C. § 112, first paragraph, as allegedly failing to comply with the written description and enablement requirements. The Office Action asserts that the claimed polynucleotide is directed to a broad genus which is allegedly not sufficiently supported by the specification and would require undue experimentation.

In response, as mentioned above, Applicants respectfully submit that in an attempt to advance prosecution and without expressing agreement with or acquiescence to the rejection, claim 21 has been amended. Applicants note that the present claims are directed to a polynucleotide encoding a polypeptide comprising the following polypeptide sequences: 1) two sequences of F46L YFP; 2) at least one calmodulin sequence; 3) at least one calmodulin-binding domain of skeletal muscle myosin light chain kinase; and 4) at least one linker sequence comprising 1-30 amino acids. Applicants note that the scope of the presently claimed invention is fully supported throughout the originally filed specification.

In view of the foregoing remarks and amendments, Applicants respectfully request withdrawal of the written description and enablement rejections.

**Response to rejection under 35 U.S.C. § 102(b)**

The Office Action rejects claims 21-23 under 35 U.S.C. § 102(b) as allegedly being anticipated by Nagai et al. (PNAS, 2001, Vol. 98, No. 6, pp. 3197-3202), hereinafter "NAGAI." The action asserts that NAGAI "specifically teaches a fluorescent protein comprising calmodulin, YFP mutant at F46L, a 26-residue peptide derived from the calmodulin (CaM)-binding region of the skeletal muscle myosin light-chain kinase, and a linker, GlyGlySerGlyGly."

In response, Applicants note that NAGAI does not teach all elements of the presently claimed invention. For example, NAGAI does not disclose a fluorescence indicator based on homo FRET (fluorescence resonance energy transfer) comprising two identical fluorescent molecules, i.e., two molecules of F46L YFP. Moreover, NAGAI discloses only pericams of YFP and not the full sequence of F46L YFP, as required by the presently claimed invention.

Applicants submit that because NAGAI fails to disclose all elements of the presently claimed invention, withdrawal of the anticipation rejection over NAGAI is requested.

**Response to rejection under 35 U.S.C. § 103(a)**

The Office Action rejects claims 21-23 under 35 U.S.C. § 103(a) as allegedly being unpatentable over U.S. Patent No. 5,998,204 to Tsien et al., hereinafter "TSIEN."

The Office Action asserts that TSIEN discloses all elements of the presently claimed invention except "that the fluorescent indicator of Tsien et al. comprises GFP instead of YFP mutant (Venus)." The action concludes that it would have been obvious for someone of ordinary skill in the art "to swap GFP with another fluorescent protein such as YFP mutant (Venus)."

In response, Applicants note that TSIEN does not disclose a fluorescent indicator based on homo FRET. In contrast to the presently claimed invention, TSIEN discloses an indicator based on hetero FRET, wherein the two fluorescent proteins are different compounds. Applicants note that TSIEN relates to the conventional FRET methods, which are also discussed in the present specification, at page 1, last paragraph, to page 2, first paragraph:

"However, all of the conventional FRET methods using fluorescent molecules have used two types of fluorescent molecules having different colors (spectra). Such methods have used a considerable part of a visible region for a single FRET observation. Thus, the conventional methods have been problematic in that a simultaneous observation of other fluorescent dyes is limited, for example."

Applicants submit that the present invention solves the above mentioned problem by applying homo FRET. Homo FRET has the advantage that the measurements can be made within a much lower wavelength range, and therefore allows observations of other phenomena in the unblocked visible wavelength regions. For example, in the case of hetero FRET using CFP/YFP, the excitation wavelength is 440 nm and the fluorescent wavelength range spreads

over 110 nm, from 465 nm to 550 nm (CFP: 465-510 nm , YFP: 515-550 nm). In contrast, homo FRET using YFP/YFP according to the present invention has an excitation wavelength of 500 nm with a fluorescent wavelength in the range of only 50 nm, from 510 nm to 550 nm (less than half compared to hetero FRET).

Applicants respectfully submit that there is nothing in TSIEN that would suggest the substitution as suggested by the Office. In view of foregoing, Applicants respectfully request withdrawal of the obviousness rejection over TSIEN.

### **Response to Double Patenting Rejection**

The Office Action rejects claim 21-23 on the ground of nonstatutory obviousness-type double patenting as allegedly being unpatentable over TSIEN, claims 1-21. The Examiner asserts that “although the conflicting claims are not identical, they are not patentable distinct from each other.”


In response, Applicants refer to above presented remarks in reply to the rejection under 35 C.F.R. § 103(a) over TSIEN, which clearly explain how TSIEN is directed to a different type of invention. Moreover, as also admitted by in the Office Action by the Examiner, TSIEN does not disclose or claim F46L YFP. Applicants note that F46L YFP was indeed not discovered and disclosed publicly until well after the issue date of TSIEN. As F46L YFP was not discovered until well after the issuance of TSIEN, the present claims cannot be obvious solely in view of the claims of TSIEN.

Applicants respectfully request withdrawal of the double patenting rejection.

**CONCLUSION**

In view of the foregoing, it is believed that all of the claims in this application are in condition for allowance, which action is respectfully requested. If any issues yet remain which can be solved by a telephone conference, the Examiner is respectfully invited to contact the undersigned at the telephone number below.

Respectfully submitted,  
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January 15, 2009  
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